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Use of Activity Measures in Behavioral Toxicology

by Lawrence Reiter*

Locomotor activity measurements have been used extensively to evaluate chemically-induced changes in CNS function. This paper focuses on several factors including apparatus, age, biological rhythm, and social setting, which influence both locomotor activity levels *per se* and chemically induced changes in these activity levels. These data illustrate that failure to recognize, specify, and consider these factors is likely to result in equivocal studies subject to possible misinterpretation.

Introduction

An overwhelming research demand currently exists in the area of environmental toxicology. The need for toxicological data on compounds currently found in our environment as well as on new materials being introduced into the environment places an increasingly heavy burden on our research capacity. In the field of behavioral toxicology, an area in its infant stages of development, the demands have preceded maturation such that the number of compounds with either known or potential neurotoxicity has continued to grow in the face of only moderate levels of toxicity testing.

This disequilibrium between the introduction of toxic materials on the one hand and toxicity testing on the other has exacerbated the need for testing procedures which are not only sensitive indicators of toxicity but are also simple, rapid and inexpensive to perform. Locomotor activity has been suggested as one such behavioral endpoint (1, 2).

In general, locomotor activity in rodents has been extensively studied in both behavioral pharmacology and behavioral toxicology. The reason for this popularity is probably twofold. First, locomotor activity occurs naturally. Most animals, for example, explore their environment and react to various components of that environment including the presence of other animals. Although the patterns of these behaviors are often unique to the particular species being studied, they are generally common to

all animal species including man. They are therefore, useful in behavioral toxicology because they both reflect the functional status of the nervous system and represent behavior which is relevant to the animal's survival. The second reason for this popularity is probably based on the ease with which "some" measure of activity can be obtained. However, this logistical ease of obtaining activity data has clouded the complexity of the behavior being measured to the extent that inconsistencies exist in the literature concerning both experimental findings and interpretation of results.

Activity is not a unitary measure of behavior. Numerous motor acts, occurring either alone or in combination, constitute an animal's behavioral repertoire. The total frequency of these behaviors, including acts such as walking, rearing, sniffing, grooming, etc., all contribute to the general activity level. However, only a portion of these acts are ambulatory in nature and, therefore, only these acts will contribute to locomotor activity. Whereas some investigators, such as Norton (3) and Draper (4), have quantitated general activity levels, the more common approach has been to examine only locomotor activity. The relative contribution of various motor acts to any particular measurement of locomotor activity will depend on the detection method employed. Therefore, use of an activity wheel will provide a "pure" measure of locomotor activity, since only walking or running contributes to this measurement whereas "jiggle cage" activity will provide a measure of both stationary and locomotor events.

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A number of experiments have reported on "home cage" activity of the rat. One of the first studies to extensively examine home cage activity was published by Richter in 1922 (5). Using an early model of the jiggle cage, complete with kymograph recordings, Richter studied the influences of food, temperature, age, and illumination on motor activity. Draper (4) also examined the influences of food or water deprivation and age on home cage activity but used an extensive catalog of 33 behaviors (both ambulatory and nonambulatory). However, relatively few experiments in the area of behavioral toxicology report long-term measurements of home cage activity. A notable exception has been the use of running wheel activity; a rather special case in which a running wheel is either attached to or becomes the home cage environment. A variety of pollutants, including automobile exhaust (6) and lead (7), have been examined by using running wheel activity.

Other investigators have used complex environments to measure locomotor activity. Barnett et al. (8, 9) employed a "plus" maze to study the effects of early undernutrition on locomotor activity, and Norton et al. (10) employed a residential maze to describe activity levels in rats with brain damage from x-irradiation, carbon monoxide, or pallidal lesions. Both these mazes consist of a series of interconnected alleys equipped with a number of photodetecting devices. Animals caged either alone or in groups are placed in this environment, and locomotor activity is measured as photocell interruptions. However, even in these experiments in which extended "home cage" activity was recorded, the activity testing generally followed the exposure period. Few experiments exist in the literature in which measurements of activity are taken throughout the exposure period (6).

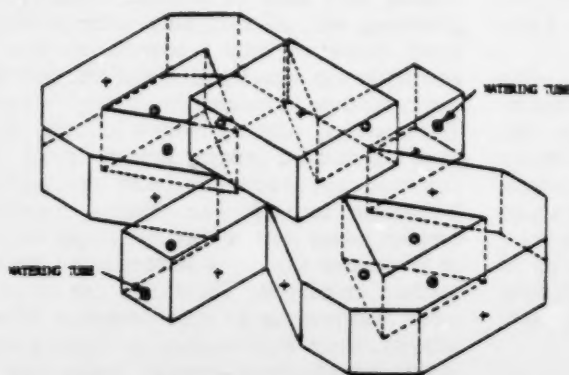


FIGURE 1. Schematic representation of residential maze used to measure locomotor activity. Ambulation is detected by eight phototransistor (+)infrared light-emitting diode (○) pairs.

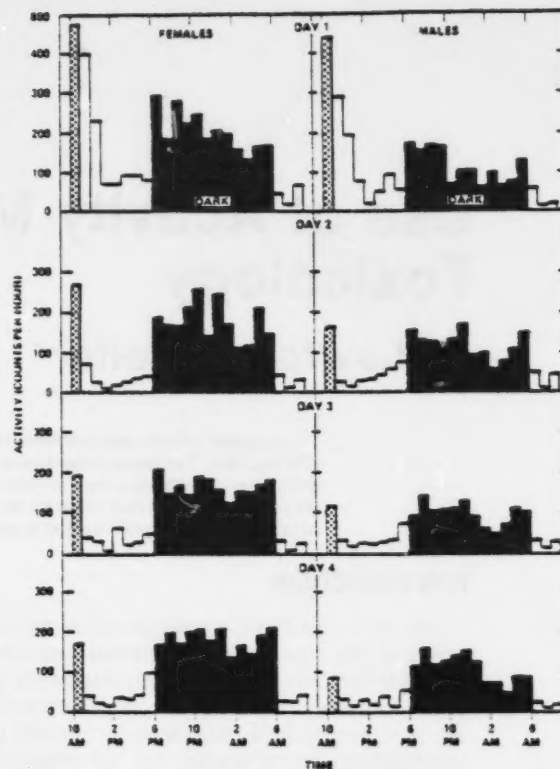


FIGURE 2. Interval histograms representing daily activity counts for six groups of three adult Sprague-Dawley rats in the residential maze. Data collected for 23 hr per day over 4 consecutive days.

When measurements extend through several daily activity cycles, various components of this behavior can be identified. Reiter et al. (11), using the residential maze (Fig. 1) to evaluate the behavioral effects of lead exposure in rats, distinguished between several types of locomotor activity based primarily on the time period during which the data were collected. Figure 2 represents residential maze activity of adult rats collected over four consecutive days. Animals were removed from the mazes for 1 hr/day (between 9:00 AM and 10:00 AM). As indicated by the cross-hatched bars in Figure 2, this procedure resulted in a burst of activity (termed exploratory activity) during the first hour in the maze. For both male and female rats, this exploratory activity is considerably higher than the remaining diurnal activity. Since most behavioral experiments utilize short-term measures of activity, they will include some component of exploratory activity, even with a repeated measures design. In addition, a treatment effect may be manifested during different periods. For example, Norton et al. (10) reported that lesions of the globus pallidus in

rats produced increased activity which was manifested primarily in the nocturnal period.

A number of factors influence the measurement of locomotor activity of the rodent. These factors, in turn, will influence the design and interpretation of toxicological studies. Four important factors were selected for discussion, namely: Apparatus, biological rhythm, age, and social setting. This list is neither meant to be all-inclusive nor is the discussion of each factor meant to be totally comprehensive. Instead, the intent is to illustrate, using selected papers, the ways in which these factors may impact on the results and interpretation of toxicological studies. Examples will emphasize experiments on rats because of their extensive use in toxicology.

Apparatus

A number of different devices used to measure motor activity have been reviewed by Finger (12). Each apparatus is characterized by its environmental complexity and the method used to detect activity. Environmental complexity includes features such as size, shape, levels of illumination and noise, color, etc. Walsh and Cummins (13) have reviewed the influence of these environmental factors on open-field behavior.

As previously indicated, the method used to record activity will influence the measurement since each method may detect different types of behavior. Also, the kinesthetic feedback to the animal and the level of effort required by the animal will depend on the device used to measure activity. Movement of a jiggle platform will provide more feedback to the animal than an interruption of an infrared light-emitting diode, and rotating a wheel will require more effort than walking on a flat surface.

Tapp et al. (14) compared seven different measures of motor activity in rats. Their results showed a general lack of correlation between the various

measures of activity (Table 1). When two measurements were taken within the same apparatus (i.e., the circular field and the light-contingent bar pressing) a significant correlation was found. A significant correlation was also observed between light-contingent bar pressing activity in a photocell cage and the activity in a revolving drum. The authors attributed these correlations to the fact that in each case the response being measured produced sensory feedback to the animal. (Photocell interruptions produced an audible click of a relay.) Otherwise, there was generally a poor correlation of behavior, such that a normal animal showed different relative levels of activity in the different test environments.

Physiological manipulations can also be shown to produce activity changes which are apparatus dependent. Tapp (15) used four different devices to measure activity of rats which were either food deprived or fed *ad libitum*. Food deprived animals were adapted on a diet of 13 g of powdered Purina Chow per day for 12 days and were tested just prior to their established feeding time. The results (Fig. 3) show that these measures were differentially affected by food deprivation. In the Williamson cage (a type of jiggle cage), there was no measurable effect of food deprivation on activity, whereas light-contingent bar pressing was increased by food deprivation and activity measured in both the photocell and circular field decreased under this same condition. Therefore, a simple physiological manipulation such as food deprivation produced changes in activity which depended on the apparatus. In fact, if only one of the devices were used, the authors would have reported either an increase, decrease, or no change in activity. Similar divergent effects resulting from food deprivation were reported by Strong (16).

Experimentally induced brain damage is another manipulation reported to produce apparatus-dependent changes in activity. Capobianco and

Table 1. Matrix of intercorrelations between activity measures (N = 40).^a

Test number	Test	1	2	3	4	5	6	7
1	Williamson cages		-0.03	0.11	0.18	0.19	0.06	0.06
2	Photocell cages			-0.02	-0.01	0.11	0.14	0.32 ^b
3	Activity wheel				0.15	0.19	-0.04	0.38 ^b
4	Circular field (alternations)					0.90 ^c	0.00	0.05
5	Circular field (total counts)						-0.01	0.10
6	Light-contingent bar pressing (total counts)							0.33 ^b
7	Light-contingent bar pressing (preference)							

^a Data of Tapp et al. (14).

^b $p < 0.05$.

^c $p < 0.01$.

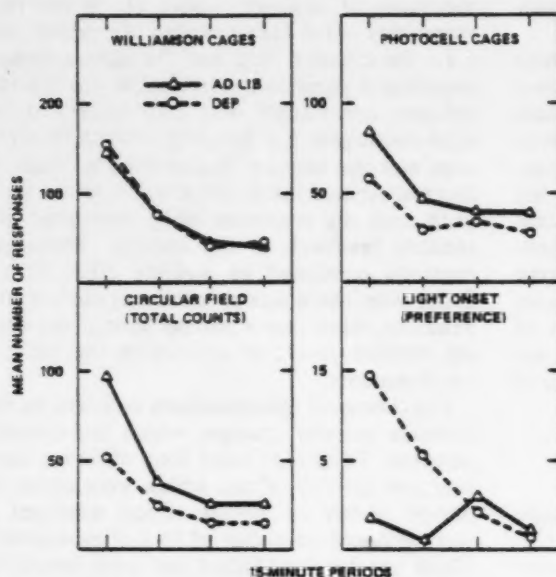


FIGURE 3. Activity counts for food deprived and *ad libitum* rats in four different measurement devices plotted over each 15-min period of 1-hr session. Data of Tapp (15).

Table 2. Effects of interruption of limbic system pathways on different activity measures.^a

Surgical Group	Running wheel	Stabilimeter	Open field	Rearing
Fornix transection	↑	↑↑	— ^b	—
Diagonal band transection	↑↑	↓	— ^c	—
Medial forebrain bundle transection	↑↑	—	—	—

^a Data of Capobianco and Hamilton (17).

^b Some evidence for transient increase.

^c Some evidence for transient decrease.

Hamilton (17) employed several activity measures following surgical interruption of various limbic system pathways in the rat. Lesions were placed in the fornix, diagonal band, and the medial forebrain bundle. Table 2 summarizes their results. All three lesions produced an increase in locomotor activity when measured in the running wheel. On the other hand, activity in a stabilimeter either increased, decreased or showed no change depending on the site of the lesion. Finally, measurements of both open-field ambulation and rearing appeared to be insensitive to these lesions. Therefore, any conclusions concerning the behavioral effects produced by lesions of the diagonal band would be either incorrect or incomplete if only one measure of activity were taken. Obviously an increase, decrease or no change in activity has meaning only with regard to a particular test environment.

This experiment suffers from one possible source of experimental error which was recognized by the authors: stabilimeter testing always preceded open-field testing. It is possible that an "order effect" of testing could account for the lack of a treatment difference in the open field. If, for example, lesions of the diagonal band altered the animal's reactivity to a novel environment, then a "carry over" effect from stabilimeter to open field would "wash out" any effect on the latter. Nevertheless, the differences obtained under these circumstances serve to further illustrate the complexity of activity measurements.

Finally, animals treated with various drugs or toxicants have been shown to respond differently when tested in different activity devices. Reiter et al. (18) reported changes in activity of male rats exposed to the pesticide Kepone which were apparatus-dependent (see Fig. 4). Following three weeks of treatment, rats showed a dose-related increase in locomotor activity in the residential maze. Conversely, when these animals were tested in an open field, a dose-related decrease in activity was recorded. It is likely that both test systems used in this experiment were measuring ambulation and, therefore, the difference in behavior resulted from differences in the animals' response to the two environments.

Stewart (19) reported that scopolamine-treated rats had a higher level of ambulation than control rats when tested in a simple open field (36 in. × 36 in.). On the other hand, scopolamine-treated animals were less active than controls when the complexity of this environment was increased by inserting partitions which divided the field into either

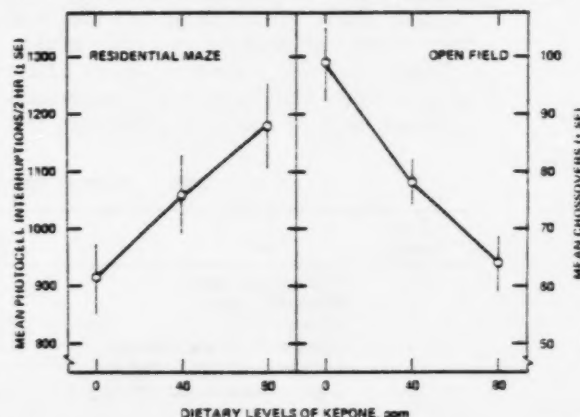


FIGURE 4. Effects of Kepone exposure (3 weeks) on two measures of locomotor activity in the rat. Kepone exposure produced a dose-related increase in locomotor activity in a residential maze but resulted in a decreased activity in an open field. Data of Reiter et al. (18).

two or four compartments. These results again demonstrate that environmental complexity will influence a drug response.

In summary, the apparatus used to measure activity will have a considerable influence on the results and interpretation of an experiment. Not only is normal activity, measured by various commonly used devices, poorly correlated, but the activity following various experimental manipulations resulting in physiological changes (e.g., food deprivation), brain damage, or chemical insult will differ, depending upon the measurement employed.

There are at least two probable sources for these apparatus-dependent differences in locomotor activity. First, various devices employ different methods of detection. A jiggle cage measures different components of motor activity than a running wheel. If a toxicant differentially alters various components of motor activity and if these components are differentially measured by any two devices, then conflicting results will be obtained. Norton (3) recorded the general activity of rats using time-lapse photography and found that amphetamine administration produced a differential effect on various motor items. It is possible that a variety of toxicants will differentially affect various components of motor behavior and therefore produce apparatus dependent changes in activity. In this respect, Krsiak et al. (20) have discussed the uses and limitations of photocell-activity cages for assessing the effects of drugs.

The second probable source for these differences in activity is the test environment and its influence on the animal. Since a number of environmental factors are known to influence activity levels [e.g., size and illumination (21) and spatial complexity (19)], the finding that environmental complexity interacts with drug- (19) and toxicant-induced (18) activity changes illustrates the need to consider these interactions in behavioral toxicity testing.

Finally, in light of all of the findings just discussed, it may be advantageous to use at least two different activity measures for initial toxicity testing. Certainly, extreme caution should be used in extrapolating data from one apparatus to another or in extrapolating limited experimental data to a general concept of activity. In fact, any use of the term activity as a unitary behavior should be discouraged.

Biological Rhythms

Both chronopharmacology and chronotoxicology have evolved from the area of chronobiology. A recent review by Reinberg and Halberg (22) introduced these terms for studies which are concerned

with the effects of either drugs or toxicants on biological rhythms and vice versa.

It is well established that different biological systems, including various metabolic pathways, show rhythmic changes in activity which influence either the pharmacodynamics of a chemical or the sensitivity of an organism to that chemical (23, 24). Attempts to determine dose-response relationships may be markedly confounded by these biological rhythms especially if their influence is unrecognized. Figure 5 is taken from a report by Pauly and Scheving (25), to illustrate the influence of the circadian rhythm on barbiturate-induced sleep time. Rats given a single dose of pentobarbital (35 mg/kg) showed marked differences in their sleep time (approximately 30%) depending on the time of day when the drug was administered. Therefore, the behavioral response (sleep in this case) to pentobarbital was rhythmic.

Müller (26) reported on the chronotoxicity of phenobarbital. When rats were given the LD₅₀ (190 mg/kg IP) of this depressant, mortality varied from 0% to 100% depending on the time of day when the drug was administered. The maximum lethality oc-

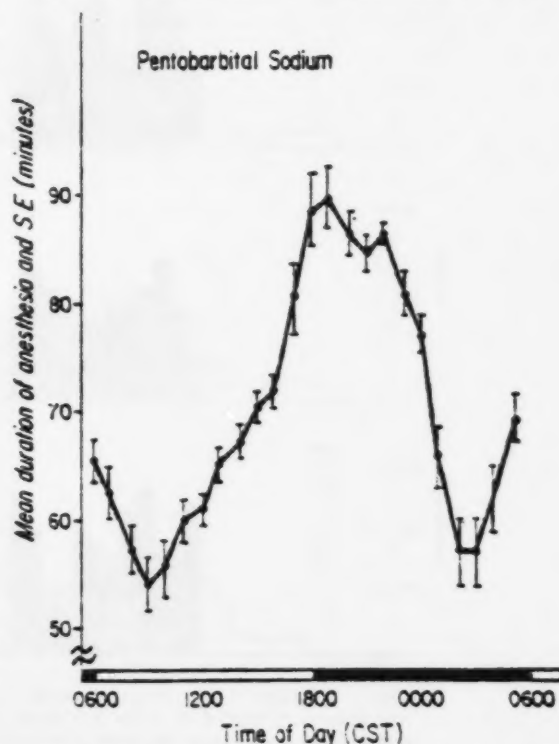


FIGURE 5. Circadian rhythm in the anesthetic duration (sleep time) following administration of 35 mg/kg pentobarbital. Data of Reinberg and Halberg (22), redrawn from original report by Pauly and Scheving (25).

curred during the diurnal period. In a subsequent experiment, the sleep time of rats following hexobarbital was compared to *in vitro* hexobarbital metabolism. These two parameters were inversely related. So, the variation in sleep time was accounted for, at least in part, by the ability of microsomal enzymes to metabolize the compound. Rhythmic changes in CNS excitability may also contribute to changes in the behavioral response. The low excitability of the nervous system during the diurnal period may be additive with the depressant effect of a barbiturate resulting in a greater toxicity during this period.

Also of concern is the influence a chemical may exert on a biological rhythm. The rat is a nocturnal animal and shows a marked circadian rhythm in activity. Figure 6 is a composite of locomotor activity data taken from three experiments in the literature. Data in Figure 6A are from Norton et al. (27) and

represent residential maze activity of a group of four adult female rats. Animals were placed in the maze daily at 10:00 AM and removed at 9:00 AM the following day. This procedure resulted in an initial burst of activity followed by a period of quiescence. With the onset of the nocturnal period, activity levels increased showing various activity maxima during this period. Figure 6B is redrawn from Barnett and Cowan (28) and represents the mean activity (measured as visits to the arms of a plus maze) of five rats. Since animals were continuously resident in this maze, no exploratory burst of activity was present. The data has been rearranged to fit the time scale of the other two data sets. Again, activity is low during the diurnal period and high at night with several nocturnal activity maxima. Finally, Figure 6C is taken from Reiter et al. (11) and represents five groups of three adult male rats. Testing was identical to that carried out by Norton et al. (27) so animals showed an initial burst of activity and, once again, short-term fluctuations (ultradian rhythm) in nocturnal activity were observed.

Repeated daily testing may be required to uncover the nocturnal ultradian rhythm seen in Figure 6. The residential maze activity shown in the left column of Figure 7 was obtained over four consecutive days. Activity during each hourly interval is presented as a percentage of the mean total daily activity for five groups of three male rats. The activity counts for each hourly interval were converted to this percentage by dividing each value by the mean hourly activity and then multiplying by 100. Each curve, therefore, represents the animals' relative daily distribution of activity.

When animals were initially placed in the maze, there was a high level of activity. As a result, activity during the nocturnal period of the first day seldom reached the average hourly activity level (100%). On subsequent days, the exploratory peak diminished and nocturnal activity became dominant. Also, a triphasic distribution of nocturnal activity developed over the 4-day period and was clearly present by the fourth day. This ultradian rhythm had a periodicity of approximately 4 hr.

Administration of *d*-amphetamine (2.0 mg/kg) 20 min prior to testing to animals established in the maze, resulted in an increased activity (see right column of Fig. 7). This drug-induced elevation of the initial activity resulted in a relative suppression of the nocturnal activity to a level below the daily average; however, it had no effect on either the ultradian rhythm or the absolute level of nocturnal activity. Recovery of normal diurnal/nocturnal activity is complete within a day along with a clear triphasic distribution of nocturnal activity.

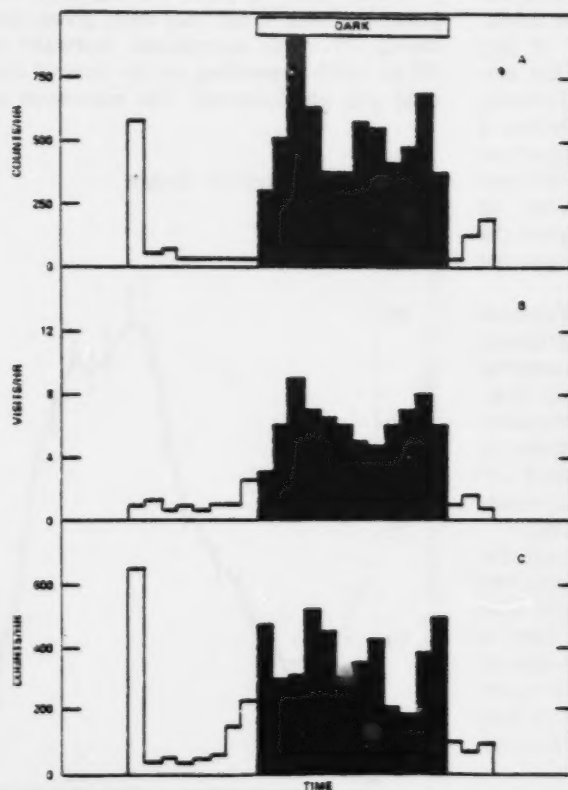


FIGURE 6. Circadian rhythm in maze activity of the rat: (A) time-interval histogram of residential maze activity for a group of four adult female rats taken from Norton et al. (27); (B) the mean activity of five rats in a "plus maze", taken from Barnett and Cowan, (28); (C) the mean of five groups of three adult males in a residential maze, taken from Reiter et al. (11). Nocturnal activity predominates showing various activity maxima during this period.

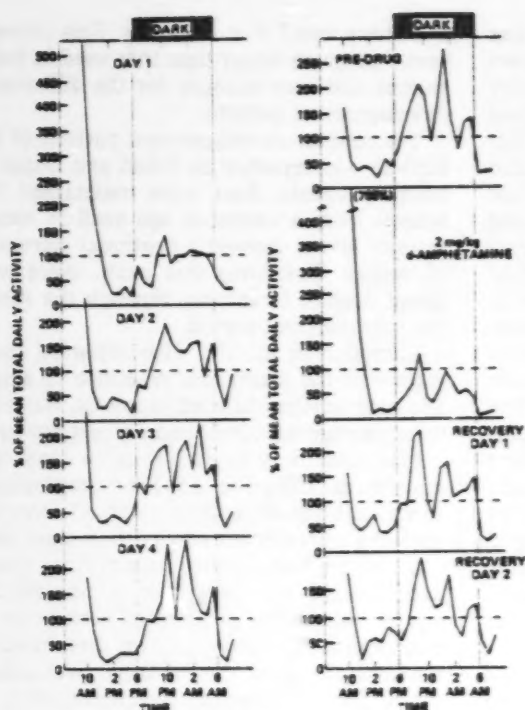


FIGURE 7. Residential maze activity in the rat. Activity is presented as a percentage of the mean total (hourly) daily activity and therefore represent the relative distribution of the animals' activity. Data represents the mean for five groups of three adult male Sprague-Dawley rats. On day 1, exploratory behavior is the major component of the activity whereas on subsequent days the nocturnal activity predominates. By day 4, a clear ultradian rhythm has developed during the nocturnal period. Administration of *D*-amphetamine (right panel) shifts the activity distribution to a pattern similar to day 1 but the ultradian rhythm persists.

Reiter et al. (11) reported that exposure to lead disrupted the nocturnal ultradian rhythm. Figure 8 shows the predicted curves for various groups of adult male rats. Activity counts were subjected to $\log_{10}$ transformations to correct for heterogeneity of variance. In control animals, the triphasic distribution of activity was clearly present. Statistical analysis (ANOVA) indicated that time accounted for a highly significant ($p < 0.0001$) 71% of the variability in activity. In the pair-fed control (a group which was used to control for the reduced food intake seen in the lead-exposed group), there was some depression of the later peaks but there was still a significant ($p < 0.03$) contribution of time to the group's activity accounting for 27% of the variability. Finally, the curve for lead-exposed animals was statistically flat ($p = 0.83$) with time accounting for less than 10% of the variability in activity. A comparison of the total nocturnal activity levels

across treatment groups showed that neither lead exposure nor early undernutrition (pair-fed controls) affected overall activity levels. Lead exposure, therefore, suppressed the ultradian rhythms without changing the overall activity level.

In summary, biological rhythms can be shown to influence an organism's response to chemicals. The time of day when testing is performed is therefore an important consideration in behavioral toxicity studies. In addition, the rhythms themselves may be altered by toxicants. These two types of interactions warrant further consideration of chronotoxicology in future research.

Age

Since locomotor activity levels show maturational changes, the possibility exists that alterations in central nervous system function resulting from perinatal exposure to pollutants could alter the developmental sequence of activity. It is also possible that perinatal exposure to chemicals may produce changes which are manifested only during certain periods of development (29).

Campbell et al. (30) reported on the development of locomotor activity in the rat. Testing was performed at various ages (each animal was tested only

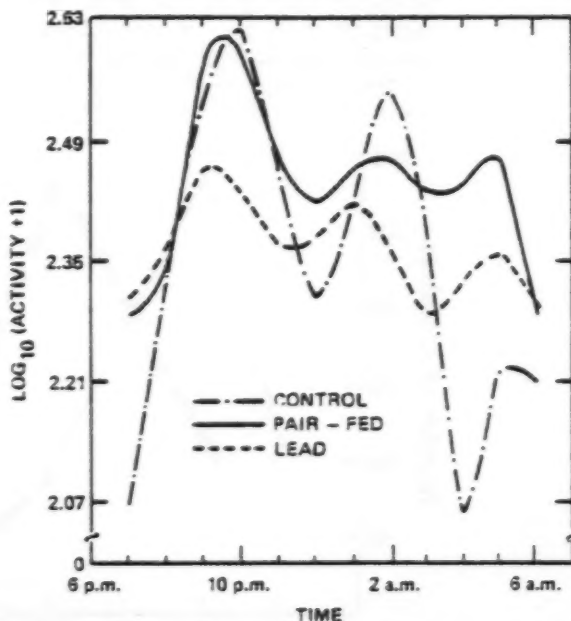


FIGURE 8. Predicted nocturnal activity curves for control and lead-treated adult male rats, tested in groups of three. $\log_{10}$ transformations were performed to correct for heterogeneity of variance. An analysis of variance indicated an overall treatment $\times$ time interaction ($p < 0.05$) demonstrating that the curves are not parallel. Data of Reiter et al. (11).

once) in a stabilimeter which was scaled to the size of the animal. The solid line in Figure 9 is redrawn from their original data and shows that activity levels peak between 10 and 20 days of age. These investigators attributed this peak in activity to dissimilar maturational rates of hindbrain facilitory and forebrain inhibitory systems. They proposed that maturation of the facilitory systems occurs first and therefore activity levels are high. As the inhibitory systems mature, activity levels decline. A similar developmental curve was reported by Melberg et al. (31) using a repeated measures design in an automated open field (Motron Electronic Mobility Meter). Therefore, the shape of this maturation curve is not solely a function of the apparatus. The broken line in Figure 9, added to the original figure, illustrates a hypothetical developmental pattern which could occur in animals showing a chemically induced delay in CNS development. Such a delay would shift the developmental curve to the right. Measurements taken at or about the age of weaning would then indicate a treatment-related hyperactivity but one which would disappear as the animals matured.

However, different developmental patterns of locomotor activity have been observed. Candland and Campbell (32) reported a steady increase in the locomotor activity of rats tested in an open field beginning at 20 days of age. Animals were tested only once and activity reached an asymptotic level at approximately 40–50 days of age. It should be noted, however, that the circular open field used in

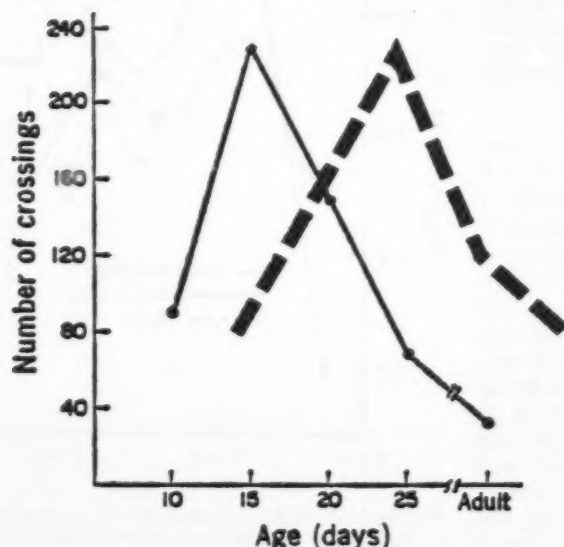


FIGURE 9. Spontaneous activity as a function of age in the rat: (—) original data, redrawn from Campbell et al. (30); (---) a hypothetical developmental curve in animals with a toxicant-induced delay in CNS development.

this study was 7 ft in diameter. This cross-sectional area was much larger than that used in the previous studies and may account for the differences in the developmental pattern.

Yet another developmental pattern of locomotor activity was reported by Smith and Dugal (33) using running wheels. Rats were maintained in running wheels from 6 weeks of age until 36 weeks. Daily activity levels showed a continual increase through 13 weeks. Following this peak, there was a continual decline in activity through the remainder of the experimental period.

Campbell et al. (30) also reported that the ontogeny of the locomotor response to amphetamine and scopolamine differed in the rat. Responsiveness to amphetamine developed earliest producing an increase in activity beginning at 10 days of age. The maximum effective dose of amphetamine was somewhat age-dependent, with 15 day-old animals showing a greater sensitivity than older animals. On the other hand, the locomotor response to scopolamine was only seen in animals 20 days of age or older. This differential sensitivity to a sympathomimetic amine on the one hand and to a cholinolytic agent on the other was taken as evidence for a differential development of these two biochemical pathways. It also serves to illustrate how age can influence the locomotor response to chemicals.

Toxicants have also been shown to produce age-dependent changes in locomotor activity. Figure 10 shows how activity varies as a function of age in rats exposed postnatally to lead (34). Mothers received 5% lead carbonate in the diet for 16 days starting at parturition. Lead was then removed from the diet and added to the drinking water in a concentration of 50 ppm. Lead-exposed animals tested in a jiggle cage showed an initial elevation in activity, but this elevation in activity disappeared as the animal matured.

Culver and Norton (35) reported juvenile hyperactivity in rats exposed postnatally to carbon monoxide (CO). At 5 days of age, they exposed rats to concentrations of CO sufficient to produce coma and respiratory failure. Hyperactivity, as measured in a residential maze, was present in these animals between 4 and 8 weeks of age, but recovery had occurred by 3 months. When adults were exposed to CO, a mild hyperactivity occurred which was not reversible. These results demonstrate that both age of exposure and age of behavioral testing are important factors for consideration in behavioral toxicity testing.

An opposite effect of age was reported following perinatal exposure to the chlorocarbon insecticide Mirex (34). Rats exposed postnatally to Mirex via the mothers' milk were reported to develop ele-

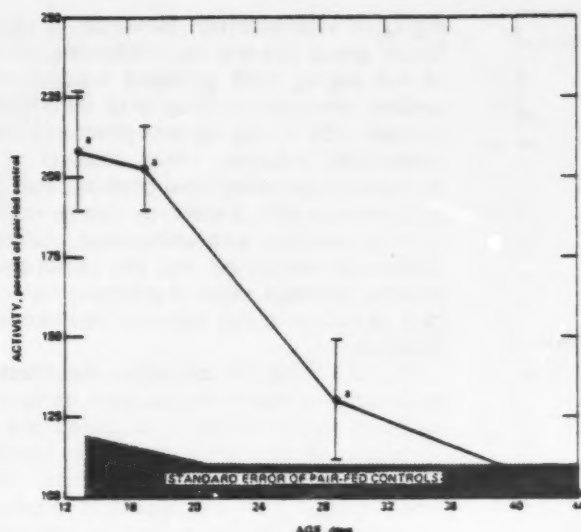


FIGURE 10. Effects of lead administration on acute exploratory activity in the rat. Values are expressed as % of pair-fed controls. Each point represents the mean activity ($\pm$ SE) for five litters (two animals/litter); (a) indicates significantly different from pair-fed controls. (*t*-test, $p < 0.05$). Data from Reiter (34).

vated locomotor activity which was manifested only as this animal matured. Residential maze activity of Mirex-exposed animals was similar to controls at 21 days of age but was elevated at both 42 and 120 days of age. Activity was elevated only during the initial period of testing so that once the animals became "established" in the maze, activity was normal. This latter finding shows that Mirex exposure produced a change in the animals' reactive locomotor activity rather than a change in spontaneous activity.

In summary, the experiments cited above demonstrate the importance of considering age in experiments employing activity measurements to study CNS toxicity. Not only do activity levels change as a function of age but chemically-induced changes in this behavior also show age-dependency. Although activity is clearly subject to maturational processes, the exact relationship between age and locomotor activity is not clear. Further studies are required to resolve the differences in the developmental course for activity reported in the literature. Finally, in studies employing perinatal exposure to pollutants, there is a definite need for longitudinal testing to uncover possible age-dependent changes in activity.

Social Setting

It has been recognized for many years that social setting can markedly alter toxicity. In 1946, Chance

(36) demonstrates that differences in group size (ranging from 2 to 32 mice per cage) produced significant differences in the toxicity of sympathomimetic amines. In his experiments, mortality increased with increasing group size. Wilson and Mapes (37), on the other hand, reported that the gastric ulcerations induced by phenylbutazone were more severe in isolated rats than in animals caged either two or four per cage. Because individuals exposed to environmental pollutants do not live in isolation, the influence of social setting (including group size) on toxicity is of practical significance.

The following experiment was performed to determine the influence of group size on locomotor activity. Either male or female adult rats were tested in residential mazes for a period of 8 days in group sizes ranging from one to four. Animals comprising a test group were housed in groups of four prior to testing and so testing *per se* did not create a new group. Residential maze activity is presented in Figures 11 and 12 as activity per rat to correct for

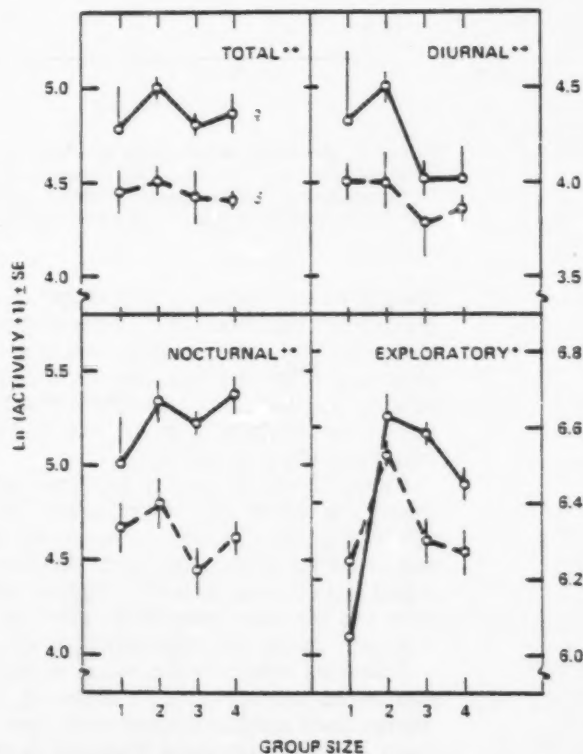


FIGURE 11. Residential maze activity as a function of group size on the first day of testing. Data is presented as activity per rat in order to correct for group size. Each point represents the mean for six groups of animals and statistical comparisons were performed using a regression analysis. The asterisk (*) denotes a significant effect of group size, $p < 0.007$; the double asterisk (**) shows a significant sex difference, $p < 0.003$.

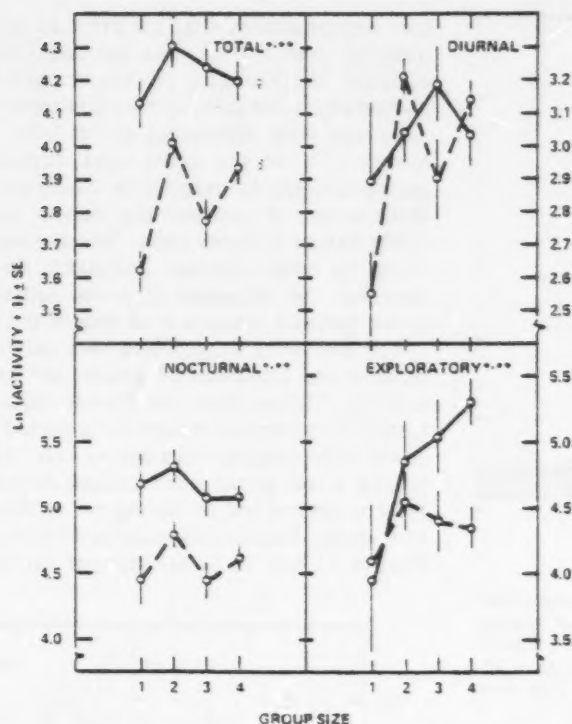


FIGURE 12. Residential maze activity as a function of group size on the fourth day of testing. Data presented as in Figure 11. The asterisk (*) shows significant effect of group size, $p < 0.04$; the double asterisk (**) indicates significant sex difference, $p < 0.01$.

the group size. On day 1, the number of animals per group had a significant effect on activity only during the initial exploratory period (first hour) whereas a clear sex difference was seen in total and diurnal activity (see Fig. 11). It appeared that for this particular environment a group size of two had the maximum effect on activity.

Figure 12 shows activity for these same animals during the fourth day in the mazes. A significant effect of group size was observed for activity obtained during all periods except the diurnal period. Again activity was generally highest with two animals and the most pronounced effect of group size was seen during the exploratory period.

Following 4 days in the maze, animals were administered amphetamine in doses of 2.0 and 4.0 mg/kg. Each animal received each dose of the drug (with order of treatment balanced across groups) with a 2-day recovery period between treatments. Figure 13 shows the activity during the first 6 hr following amphetamine administration. (Day 4 is presented to illustrate the predrug levels.) Group size had no effect on the locomotor response to 2.0

mg/kg of amphetamine. However, a significant effect of group size was seen following administration of 4.0 mg/kg with grouped animals showing a greater response to drug than individually tested animals. The 4.0 mg/kg dose produced considerable stereotypic behavior which consists of repetitive movements including head bobbing, sniffing, licking, and chewing (38). Stereotypy can be sufficiently severe to interfere with ambulation. Although quantitation of stereotypy was not performed in these studies, the main effect of grouping was observed to be a reduction in this behavior and hence more ambulation.

File and Pope (39) examined the effects of chlorpromazine (a major tranquilizer) on locomotor behavior of single or paired rats tested in a hole board apparatus. Both ambulation and the number of head dips were measured in each animal, tested either singly (drugged or undrugged) or in pairs in which either one, both, or neither of the animals was drugged. For undrugged animals, the social condi-

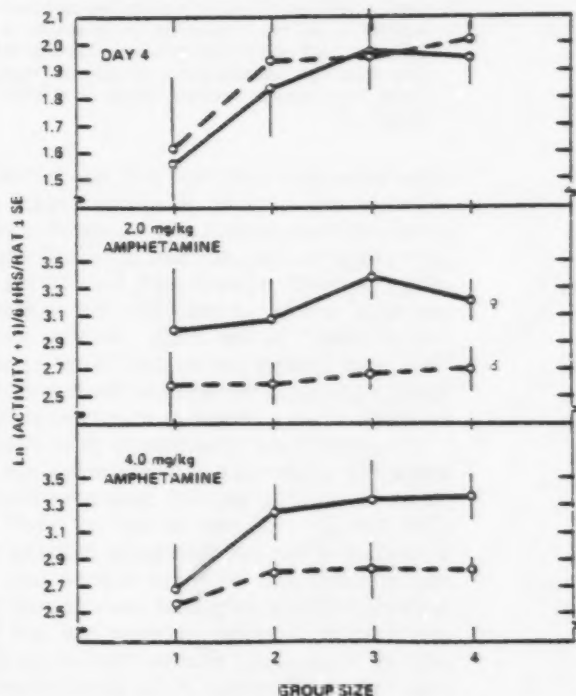


FIGURE 13. Effects of amphetamine administration on residential maze activity, as a function of group size. Activity is corrected for group size and is presented for the first six hours following amphetamine administration. The top panel (day 4) illustrates activity levels on the day before the first amphetamine injection. Group size had a significant (regression analysis, $p < 0.0003$) effect on the amphetamine response following 4.0 mg/kg with the larger groups showing a higher ambulatory response to drug.

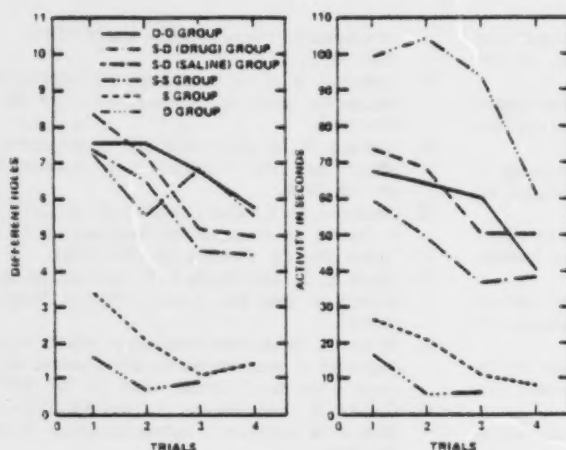


FIGURE 14. Mean number of different holes explored and mean time spent in motor activity for rats tested in a hole-board apparatus. The drug group was given 2.0 mg/kg chlorpromazine 1 hr prior to testing. Animals were tested either alone (S- and D- groups) or in pairs (S-S, S-D, D-D group) and data are presented for the two drug and two social conditions. Redrawn from File and Pope (39).

tion led to an increase in both head dipping and ambulation (Fig. 14). Chlorpromazine administration depressed both head-dipping behavior and ambulation in rats tested individually but did not depress head dipping in the groups of two. In contrast, chlorpromazine still depressed locomotor activity even when this activity was increased by the presence of another animal.

Results of these two studies indicate that normal activity is influenced by the presence of another animal resulting in an increased level of activity for animals tested in groups. The response to drugs was also altered by the presence of another animal. Dose-response relationships to a toxicant may therefore depend on the social conditions during testing. It is also possible that the qualitative response to a toxicant could differ under different social conditions.

Summary and Conclusions

In the area of neurotoxicology, there is clearly a need for sensitive indicators of toxicity which are also simple, rapid and inexpensive to perform. Locomotor activity represents one such behavioral measure. This paper focused on several factors which influence both activity levels *per se* and chemically-induced changes in these activity levels. A failure to recognize, specify, and consider these factors including apparatus, biological rhythms, age and social setting is likely to result in equivocal studies subject to misinterpretation. The multifac-

ceted character of the locomotor response forms the basis of its utility. On the one hand, we must attempt to understand better those factors which influence this behavior, thereby optimizing the power of the measurement. On the other hand, we must not be too restrictive in the experimental design diminishing the influence of these factors and therefore decreasing the sensitivity of the measurement. Attempts should be made to systematically characterize locomotor activity changes following exposure to known neurotoxic agents. If activity testing is then performed in conjunction with other measures of CNS function, the utility of this system will improve.

REFERENCES

1. Committee for the Working Conference on Principles of Protocols for Evaluating Chemicals in the Environment. Environmental Studies Board. National Academy of Sciences-National Academy of Engineering and Committee on Toxicology. National Research Council. Principles for Evaluating Chemicals in the Environment. National Academy of Sciences, Washington, D. C., 1975.
2. Second Task Force for Research Planning in Environmental Health Science. Human Health and the Environment—Some Research Needs. U. S. Depart. Health, Education, and Welfare. Public Health Service/National Institutes of Health. U. S. Gov. Printing Office, Wash., D. C., 1977.
3. Norton, S. Amphetamine as a model for hyperactivity in the rat. *Physiol. Behav.* 11: 181 (1973).
4. Draper, W. A. A behavioral study of the home-cage activity of the white rat. *Behaviour* 28: 280 (1967).
5. Richter, C. P. A behavioristic study of the activity of the rat. *Comp. Psychol. Monographs* 1: 1 (1922).
6. Gage, M. I. Automotive exhaust and mouse activity. Relation between pollutant concentrations and decreases in wheel running. *Arch. Environ. Health*, in press.
7. Hastings, L., et al. Behavioral effects of low level neonatal lead exposure. *Pharmacol. Biochem. Behav.* 7: 37 (1977).
8. Barnett, S. A., et al. An artificial habitat for recording movement. *J. Physiol.* 187: 15 (1966).
9. Barnett, S. A., et al. Early nutrition and the activity of feeding of rats in an artificial environment. *Dev. Psychobiol.* 4: 1 (1971).
10. Norton, S., et al. Comparison of the structure of hyperactive behavior in rats after brain damage from x-irradiation, carbon monoxide and pallidal lesions. *Brain Res.* 116: 49 (1976).
11. Reiter, L. W., et al. Locomotor activity measurements in behavioral toxicology: effects of lead administration on residential maze behavior. In: *Behavioral Toxicology: An Emerging Discipline*. H. Zenick, and L. Reiter Eds., GPO, Washington, D. C., 1978.
12. Finger, F. W. Measuring behavioral activity. In: *Methods in Psychobiology*. Vol. 2. R. D. Myers, Ed., Academic Press, New York-London, 1972, pp. 1-19.
13. Walsh, R. N., and Cummins, R. A. The open-field test: a critical review. *Psychol. Bull.* 83: 482 (1976).
14. Tapp, J. I., et al. Intercorrelational analysis of some common measures of rat activity. *Psychol. Repts.* 23: 1047 (1968).
15. Tapp, J. I. Activity, reactivity and the behavior-directing properties of stimuli. In: *Reinforcement and Behavior*. J. I. Tapp, Ed., Academic Press, New York, 1969, p. 146.

16. Strong, P. N., Jr. Activity in the white rat as a function of apparatus and hunger. *J. Comp. Phys. Psychol.* 50: 596 (1957).
17. Capobianco, S., and Hamilton, L. W. Effects of interruption of limbic system pathways on different measures of activity. *Physiol. Behav.* 17: 65 (1976).
18. Reiter, L. W., et al. Comparative behavioral toxicology of mirex and Kepone in the rat. *Toxicol. Appl. Pharmacol.* 41: 143 (1977).
19. Stewart, W. J. Environmental complexity does affect scopolamine-induced changes in activity. *Neurosci. Letters.* 1: 121 (1975).
20. Krsiak, M., et al. Uses and limitations of photocell activity cages for assessing effects of drugs. *Psychopharmacol.* 17: 258 (1970).
21. Nagy, L. M., and Forrest, E. J. Open-field behavior of C3H mice: effect of size and illumination of field. *Psychon. Sci.* 20: 19 (1970).
22. Reinberg, A., and Halberg, F. Circadian chronopharmacology. *Ann. Rev. Pharmacol.* 11: 455 (1971).
23. Scheving, L. E., et al., Eds. *Chronobiology*. Igaku Shoin Ltd., Tokyo, 1974.
24. Hastings, J. W., and Schweiger, G. S. The molecular basis of circadian rhythms. *Dahlem Konferenzen*, Berlin, 1975.
25. Pauly, J. E., and Scheving, L. E. Temporal variation in the susceptibility of white rats to pentobarbital sodium and tremorine. *Int. J. Neuropharmacol.* 3: 651 (1964).
26. Müller, O. Circadian rhythmicity in response to barbiturates. In: *Chronobiology*, L. E. Scheving, F. Halberg, and J. E. Pauly, Eds., Igaku Shoin Ltd., Tokyo, 1974, p. 187.
27. Norton, S., et al. Development of nocturnal behavior in albino rats. *Behav. Biol.* 15: 317 (1975).
28. Barnett, S. A., and Cowan, P. E. Activity, exploration, curiosity and fear: an ethological study. *Interdisciplinary Sci. Rev.* 1: 43 (1976).
29. Spyker, J. M. Assessing the impact of low level chemicals on development: behavioral and latent effects. *Fed. Proc.* 34: 1835 (1975).
30. Campbell, B. A., et al. Ontogeny of adrenergic arousal and cholinergic inhibitory mechanisms in the rat. *Science* 166: 635 (1969).
31. Melberg, P., et al. Ontogenetic development of locomotor activity and rate of tyrosine hydroxylation. *Psychopharm.* 49: 119 (1976).
32. Candland, D. K., and Campbell, B. A. Development of fear in the rat as measured by behavior in the open field. *J. Comp. Physiol. Psychol.* 55: 593 (1962).
33. Smith, L. C., and Dugal, L. P. Age and spontaneous running activity of male rats. *Can. J. Physiol. Pharmacol.* 43: 852 (1965).
34. Reiter, L. Behavioral toxicology: effects of early postnatal exposure to neurotoxins on development of locomotor activity in the rat. *J. Occup. Med.* 19: 201 (1977).
35. Culver, B., and Norton, S. Juvenile hyperactivity in rats after acute exposure to carbon monoxide. *Exptl. Neurol.* 50: 80 (1976).
36. Chance, M. R. A. Aggregation as a factor influencing the toxicity of sympathomimetic amines in mice. *J. Pharmacol. Exptl. Therap.* 87: 214 (1946).
37. Wilson, C. W. M., and Maper, R. E. A. The effects of group composition on drug action. In: *Animal Behavior and Drug Action*, H. Steinburg, Ed., Little Brown, Boston, 1964, p. 238.
38. Randrup, A., and Munkvad, I. Biochemical, anatomical and psychological investigations of stereotyped behavior induced by amphetamines. In: *Amphetamines and Related Compounds*, Proceedings of the Mario Megri Institute for Pharmacological Research, Milan, Italy, E. Costa and S. Garattini, Eds., Raven Press, New York, 1970, p. 695.
39. File, S. E., and Pope, J. H. The action of chlorpromazine on exploration in pairs of rats. *Psychopharmacol.* 37: 249 (1974).